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PATENT COOPERATION TREATY

From the INTERNATIONAL SEARCHING AUTHORITY

PCT

INVITATION TO PAY ADDITIONAL FEES

(PCT Article 17(3)(a) and Rule 40.1)

To:
WOLF, GREENFIELD & SACKS, P.C.
Attn. Lockhart, Helen C.
600 Atlantic Avenue
Boston, Massachusetts 02210
UNITED STATES OF AMERICA

REGISTERED

Applicant's or agent's file reference M0656/7063WO	Date of mailing *(day/month/year)* 26/10/2001
International application No. PCT/US 01/07464	**PAYMENT DUE** within 45 ~~months~~/days from the above date of mailing
Applicant MASSACHUSETTS INSTITUTE OF TECHNOLOGY	International filing date *(day/month/year)* 08/03/2001

1. This International Searching Authority

(i) considers that there are 4 *(number of)* inventions claimed in the international application covered by the claims indicated ~~below~~/on the extra sheet:

Subject to PTA? YES/NO
per docket/ECB
SMT 11-6-01

and it considers that the international application does not comply with the requirements of unity of invention (Rules 13.1, 13.2 and 13.3) for the reasons indicated ~~below~~/on the extra sheet:

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File Folder 12-5-01	
ECB 12-26-01	
Docket Entry 12-10-01	
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Annuities	
Confirmation	

(ii) [X] has carried out a partial international search (see Annex) [ ] will establish the international search report on those parts of the international application which relate to the invention first mentioned in claims Nos.:
1-27

(iii) will establish the international search report on the other parts of the international application only if, and to the extent to which, additional fees are paid

2. The applicant is hereby **invited,** within the time limit indicated above, to pay the amount indicated below:

EUR 945,00	x	3	=	EUR 2.835,00
Fee per additional invention		number of additional inventions		total amount of additional fees
Or, _____	x	_____	=	_____

The applicant is informed that, according to Rule 40.2(c), **the payment of any additional fee may be made under protest,** i.e., a reasoned statement to the effect that the international application complies with the requirement of unity of invention or that the amount of the required additional fee is excessive.

3. [X] Claim(s) Nos. see remark have been found to be unsearchable under Article 17(2)(b) because of defects under Article 17(2)(a) and therefore have not been included with any invention.

Name and mailing address of the International Searching Authority	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Mireille Claudepierre

Form PCT/ISA/206 (July 1992)

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-27

Methods for preventing proliferation of a tumor or for preventing tumor cell metastasis, comprising exposing a tumor cell to heparinase III, either native of modified.

Methods for preparing therapeutic agents, i.e., HLGAG fragments, for tumor treatment, comprising isolating of a portion of a tumor, treating it with heparinase III to produce HLGAG fragments, and isolating HLGAG fragments, possibly further comprising determining the sequence of the HLGAG fragments.

Methods for treating a subject having a tumor, comprising administering to the subject a therapeutic, synthetic or isolated HLGAG fragment, identified or produced when the tumor is contacted with heparinase III.

Pharmaceutical compositions comprising heparinase III, either native or modified, for preventing metastasis of a tumor cell, e.g., with a targeting molecule for targeting heparinase III to the tumor, the targeting molecule being a compound which binds specifically to an antigen on the surface of a tumor cell, or with an anti-cancer compound.

Pharmaceutical compositions comprising a therapeutic HLGAG fragment for preventing metastasis of a tumor cell, e.g., with an anti-cancer compound.

2. Claims: 28-49

A substantially pure heparinase III comprising a polypeptide according to SEQ ID NO:2, or having conservative substitutions thereof within residues non-essential to enzymatic function, wherein at least on His residue from the group of His36, His105, His110, His 139, His152, His 225, His234, His241, His424, His469 and His539 has been substituted with an Ala, Ser, Tyr, Thr or Lys residue.

A substantially pure heparinase III having a modified product profile which is at least 10% different than the product profile of native heparinase III.

A substantially pure heparinase III that can cleave a heparan sulfate having a modified k-cat value which is at least 10% different than a k-cat value of native heparinase III.

A pharmaceutical preparation comprising a heparinase III as said.

An immobilized substantially pure modified heparinase III

comprising a modified heparinase III as said and a solid support.

A method of specifically cleaving a HLGAG comprising contacting a HLGAG with a modified heparinase III as said, e.g., wherein the heparinase III is administered to subject in need for inhibiting angiogenesis, or wherein the heparinase is administered to a tumor, or wherein the heparinase III is adminstered in a polymeric delivery device or in a vehicle for injection or in a vehicle for topical application (e.g., to the eye), or wherein the method is a method for sequencing HLGAG fragments.

3. Claims: 50-54

Methods for treating or preventing a subject having a cancer or at risk of developing a cancer, comprising administering a therapeutic HLGAG fragment, e.g., a composition of HLGAG fragments wherein at least 50%, 75% or 90% of the HLGAG fragments are di- or tri-sulfated disaccharides, or wherein the therapeutic HLGAG fragment is free of mono- or unsulfated disaccharides.

4. Claims: 55-60

A method for preparing LMWH comprising contacting an HLGAG sample with a modified heparinase III molecule to produce LMWH.

A composition comprising LMWH produced by contacting an HLGAG sample with a modified heparinase III. Methods of treating or preventing, e.g., of a disorder associated with coagulation, or of a tumor, or of psoriasis, or of neovascularization, comprising administering to a subject a composition as said.

Motivation of the Objection against Unity

The prior art contains the following documents:
- WO9513830, disclosing and claiming the inhibitory effect of heparinase on angiogenesis, e.g., in a tumor;
- WO9201003, disclosing and claiming glycosaminoglycan derivatives and their use, e.g., in impeding the formation of tumor metastases;
- R. Godavarti et al. (1996) Biochem. Biophys. Res. Commun. 225:751-758 and WO9412618, disclosing heparinase III and its encoding gene from Flavobacterium heparinum;
- EP0244236 and EP0394971, disclosing the preparation of a low-molecular weight heparin (LMWH) by chemical or enzymatic degradation of heparin or heparan sulphate, e.g., with the help of heparinase, and its use in inhibition of angiogenesis and the treatment of tumors.

In the light of these prior art documents, a first problem underlying this application can be defined as the need for further means and methods for preventing proliferation of a tumor or for preventing tumor

cell metastasis. The solution as disclosed and claimed can be summarized as the provision of such means and methods comprising compositions containing heparinase III, or therapeutic heparin-like glycosaminoglycan (HLGAG) fragments obtained with the help of heparinase III and methods for the preparation and sequencing of these HLGAG fragments comprising the use of heparinase III, as well as the use of these compositions.

A second problem underlying the current application in view of the prior art can be summarized as the need for further heparinases. The solution as disclosed and claimed can be summarized as the provision of native or modified forms (mutants) of heparinase III, and the uses of these modified heparinases III.

A third problem underlying the current application in view of the prior art can be summarized as the need for further methods of treating or preventing a subject having a cancer or at risk of developing cancer. The solution as disclosed and claimed can be summarized as the provision of such methods comprising administering a therapeutic HLGAG fragment, or a composition of therapeutic HLGAG fragments with a defined content of di- or tri-sulfated disaccharides.

A fourth problem underlying the current application can be summarized as the need for further methods for the preparation of LMWH. The solution as disclosed and claimed can be summarized as the provision of a method for preparing LMWH comprising the use of a modified heparinase III, as well as the use of said LMWH in the preparation of pharmaceutical compositions and their use in treating or preventing disorders and diseases.

In view of the fact that methods for treating or preventing tumor proliferation or metastasis, e.g., comprising administering heparinases, glycosaminoglycans or LMWH, are known, in view of the fact that heparinase III, glycosaminoglycans and LMWH are known, and methods for their preparation, are known, in view of the different problems underlying the different solutions as disclosed and claimed, and due to the fact that no other technical feature can be distinguished which, in the light of the prior art, could be regarded as special technical features common to these solutions, the ISA is of the opinion that there is no single inventive concept underlying the plurality of claimed inventions of the present application in the sense of Rule 13.1 PCT. Consequently there is a lack of unity and different inventions, not belonging to a common inventive concept are formulated as the different subjects on the communication pursuant to Art. 17(3)(a) PCT.

The application has been divided into the above (groups of) inventions which individually are considered to meet the requirement of unity. If additional fees are paid for (one or more of) the, as yet, unsearched invention(s), the subsequent search(es) might reveal prior art which leads to a finding of lack of unity a posteriori within (one or more of) the, as yet, unsearched invention(s). Should this be the case, as a rule, no further invitation to pay additional fees will be issued. Only the first identified invention in each group of inventions, for which additional search fees have been paid in due time and which subsequently is considered to lack unity, will be searched.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 206

Continuation of Box 3.

Although claims 1-22, insofar as in vivo methods are concerned, are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

Annex to Form PCT/ISA/206
COMMUNICATION RELATING TO THE RESULTS OF THE PARTIAL INTERNATIONAL SEARCH

International Application No
PCT/US 01/07464

1. The present communication is an Annex to the invitation to pay additional fees (Form PCT/ISA/206). It shows the results of the international search established on the parts of the international application which relate to the invention first mentioned in claims Nos.:
1-27
2. This communication is not the international search report which will be established according to Article 18 and Rule 43.

3. If the applicant does not pay any additional search fees, the information appearing in this communication will be considered as the result of the international search and will be included as such in the international search report.

4. If the applicant pays additional fees, the international search report will contain both the information appearing in this communication and the results of the international search on other parts of the international application for which such fees will have been paid.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 96 01648 A (IBEX TECHNOLOGIES R AND D INC ;ZIMMERMANN JOSEPH (US); VLODAVSKY I) 25 January 1996 (1996-01-25) the whole document page 3, line 23 -page 4, line 5 page 9, line 17 -page 10, line 30 examples 3-5,9-16 claims 1-28 ---	1-27
X	NATKE B ET AL.: "Heparinase treatment of bovine smooth muscle cells inhibits fibroblast growth factor-2 binding to fibroblast growth factor receptor but not FGF-2 mediated cellular proliferation" ANGIOGENESIS , vol. 3, no. 3, 1999, pages 249-257, XP001030515 abstract ---	1-27
X	MURPHY P R ET AL.: "Basic fibroblast growth factor binding and processing by human glioma cells." MOLECULAR AND CELLULAR ENDOCRINOLOGY, vol. 114, no. 1-2, 1995, pages 193-203, XP001030507 ISSN: 0303-7207 abstract --- -/--	1-17, 23-27

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 95 13830 A (MASSACHUSETTS INST TECHNOLOGY ;CHILDRENS MEDICAL CENTER (US)) 26 May 1995 (1995-05-26) the whole document page 9, line 29 -page 10, line 33 page 13, line 7 -page 40, line 18 claims 1-21 ---	1-17, 23-27
X	WO 94 21689 A (CANCER RES CAMPAIGN TECH ;LYON MALCOLM (GB); GALLAGHER JOHN THOMAS) 29 September 1994 (1994-09-29) the whole document page 3, line 21 -page 10, line 17 table 1 claims 1-33 ---	18-27
X	WO 93 19096 A (CANCER RES CAMPAIGN TECH) 30 September 1993 (1993-09-30) the whole document page 7, line 32 -page 15, line 27 tables 1,2 claims 1-40 ---	18-27
X	WO 93 05167 A (CHILDRENS MEDICAL CENTER) 18 March 1993 (1993-03-18) page 3, line 1 -page 4, line 3 page 11, line 25 -page 16, line 33 figure 3 ---	18-27
A	WO 92 01003 A (UNIV TEXAS) 23 January 1992 (1992-01-23) the whole document ---	18-27
A	PADERA R ET AL.: "FGF-2/fibroblast growth factor receptor/heparin-like glycosaminoglycan interactions: A compensation model for FGF-2 signaling." FASEB JOURNAL, vol. 13, no. 13, October 1999 (1999-10), pages 1677-1687, XP002179630 ISSN: 0892-6638 the whole document ---	18-27
A	VENKATARAMAN G: "Sequencing complex polysaccharides" SCIENCE, vol. 286, 15 October 1999 (1999-10-15), pages 537-542, XP002179570 cited in the application the whole document --- -/--	19

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	GODAVARTI R ET AL.: "Heparinase III from Flavobacterium heparinum: Cloning and recombinant expression in Escherichia coli." BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, vol. 225, no. 3, 1996, pages 751-758, XP002179572 ISSN: 0006-291X cited in the application the whole document abstract page 751, line 15 -page 752, line 2 page 757, line 42-50 ---	
A	WO 94 12618 A (MASSACHUSETTS INST TECHNOLOGY ;UNIV IOWA RES FOUND (US)) 9 June 1994 (1994-06-09) the whole document ---	
A	LINHARDT R J ET AL.: "Examination of the substrate specificity of heparin and heparan sulfate lyases" BIOCHEMISTRY, vol. 29, no. 10, 1990, pages 2611-2617, XP002028479 ISSN: 0006-2960 cited in the application abstract page 2616, left-hand column, line 30 -right-hand column, line 15 ---	
A	ERNST S ET AL.: "Enzymatic degradation of glycosaminoglycans." CRITICAL REVIEWS IN BIOCHEMISTRY AND MOLECULAR BIOLOGY, vol. 30, no. 5, 1995, pages 387-444, XP001030549 ISSN: 1040-9238 cited in the application abstract ---	
A	AMEER G A ET AL.: "A new approach to regional heparinization: Design and development of a novel immobilized heparinase device." BLOOD PURIFICATION. MEETING INFO.: THIRD INTERNATIONAL CONFERENCE ON CONTINUOUS RENAL REPLACEMENT THERAPIES SAN DIEGO, CALIFORNIA, USA MARCH 5-7, 1998 , vol. 16, no. 2, 5 March 1998 (1998-03-05), pages 107-108, XP001032809 abstract --- -/--	

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Annex to Form PCT/ISA/206
COMMUNICATION RELATING TO THE RESULTS
OF THE PARTIAL INTERNATIONAL SEARCH

International Application No
PCT/US 01/07464

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP 0 244 236 A (NOVO INDUSTRI AS) 4 November 1987 (1987-11-04) the whole document ---	
A	EP 0 394 971 A (KABIVITRUM AB ;HARVARD COLLEGE (US)) 31 October 1990 (1990-10-31) the whole document ---	
T	BERRY D ET AL.: "Distinct heparan sulfate glycosaminoglycans are responsible for mediating fibroblast growth factor-2 biological activity through different fibroblast growth factor receptors" FASEB JOURNAL ON LINE, 6 April 2001 (2001-04-06), XP002179629 the whole document -----	18-27

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Patent Family Annex

Information on patent family members

International Application No
PCT/US 01/07464

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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		EP 0726773 A1	21-08-1996
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Patent Family Annex

Information on patent family members

International Application No
PCT/US 01/07464

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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